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Tumor Suppression by p53: Fall of the Triumvirate?

Andreas K. Hock[1] and Karen H. Vousden[1,*]
[1]The Beatson Institute for Cancer Research, Switchback Road, Glasgow G61 1BD, UK
*Correspondence: k.vousden@beatson.gla.ac.uk
DOI 10.1016/j.cell.2012.05.024

p53 is a key tumor suppressor protein that has numerous functions. Its primary mode of action has generally been ascribed to the induction of cell-cycle arrest, apoptosis, or senescence upon stress. Li et al. challenge this dogma with evidence that all three of these programs are dispensable for p53's tumor suppressive role.

Over the past 30 years, the p53 tumor suppressor has been subjected to intense scrutiny, with a bewildering and ever-increasing number of functions and activities attributed to it. A general consensus has emerged, however, that the key function of p53 in preventing tumor development is the ability to inhibit the outgrowth of inchoate cancers. An elegant and simple model built on numerous studies dictates that many of the stress signals encountered by nascent tumor cells (such as oncogene activation, telomere erosion, hypoxia, and genotoxic damage) lead to the activation of p53, which in turn drives the expression of genes that coordinate programs of three key responses: cell-cycle arrest, apoptosis, and senescence (Vousden and Prives, 2009) (Figure 1). The cell exposed to oncogenic stress is therefore prevented from further proliferation and tumor development avoided.

So the publication of a paper entitled "Tumor Suppression in the Absence of p53-Mediated Cell-Cycle Arrest, Apoptosis, and Senescence" will cause some excitement and possibly a degree of consternation in the field (Li et al., 2012). Has all our thinking so far been misled? If these three activities are not required for p53 to suppress tumor development, then what is? Are the other activities of p53—that have so far been thought of rather as support roles—really the key to cancer prevention? Certainly this very interesting study will generate much attention.

p53 is a transcription factor and acts primarily to regulate gene expression. Although much of the regulation of p53 activity is determined by the stability of the p53 protein, a large number of post-translational modifications on p53 also function to regulate DNA binding and engagement with the transcriptional machinery (Dai and Gu, 2010). In general, events such as phosphorylation or acetylation on individual sites have rather modest effects on p53 activity, and identifying key modifications that are critical for p53 function has proven to be rather frustrating. Now Li et al. provide evidence that a p53 protein mutated in three of the lysines that are subject to acetylation in the wild-type protein (the 3KR mutant) fails to induce cell-cycle arrest, apoptosis, or senescence in mice (supporting results from the same group showing acetylation is important for these three p53 functions in cells)—but yet retains the ability to protect mice from tumor development.

Maybe this should not be so surprising; indeed several previous studies have hinted that not all these responses are always required (Bieging and Attardi, 2012). Loss of the primary mediators of p53-induced cell-cycle arrest (p21) or apoptosis (PUMA) clearly do not to lead to tumor susceptibility in the same way as loss of p53. Other p53 mutants that are defective in apoptosis or cell-cycle arrest have been shown to retain tumor suppressor activity (Brady et al., 2011; Liu et al., 2004), although in these cases at least one of the "big three" responses was retained. Intriguingly, the p53-mediated induction of arrest and apoptosis that is seen in the immediate response following irradiation was shown to be irrelevant for suppression of radiation-induced lymphoma (Christophorou et al.,

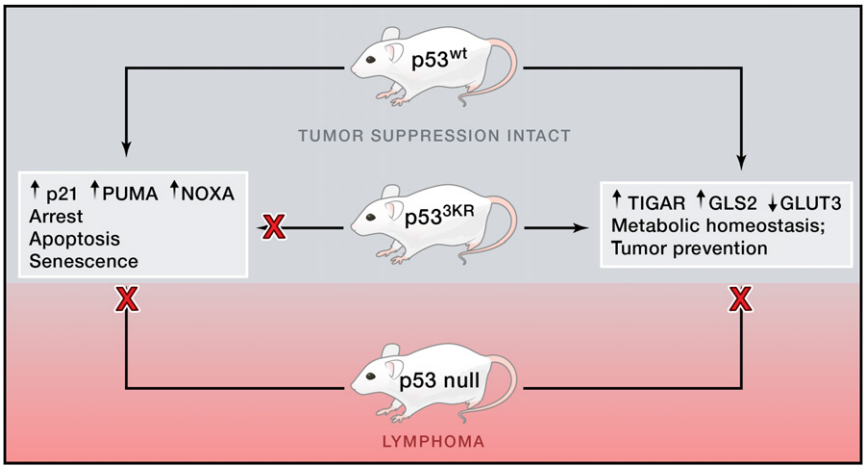

Figure 1. Cell-Cycle Arrest, Senescence, and Apoptosis are Dispensable for p53's Tumor Suppressive Potential

Mice with wild-type p53 are capable of inducing many target genes, including p21, BAX, PUMA, and NOXA to drive cell-cycle arrest, apoptosis and senescence. p53 also positively or negatively regulates the expression of genes like TIGAR, GLS2 and GLUT3 that control metabolism and antioxidant defenses. Complete loss of p53 results in a defective ability to engage these programs and results in spontaneous development of lymphomas in mice. Mutating three lysines to arginine (K117R, K161R, K162R) to inhibit acetylation at these sites renders p53 incapable of inducing cell-cycle arrest, apoptosis, and senescence. Surprisingly this does not change p53's ability to regulate the expression of metabolic target genes or prevent its tumor suppressive action.

2006). Finally, reactivation of p53 in established tumors results in tumor regression that is accompanied by either apoptosis or senescence, depending on the tumor type (Ventura et al., 2007). Taken together, these studies have shown that cell-cycle arrest, senescence, and apoptosis are not all necessary for tumor suppression but generally suggested that at least one or other of these responses would be important. Li et al. now show that none of them is actually required—at least to restrain lymphoma development.

Delving into the exact nature of the defect in this mutant, the authors show that although the 3KR mutant retains DNA binding activity, there is a differential loss of transcriptional function, so that certain key mediators of the cell-cycle arrest or apoptotic response to p53—such as p21 or PUMA—cannot be induced by the lysine mutant. In contrast, other transcriptional targets of p53, including MDM2, remain as responsive as to the wild-type p53 protein. Differential regulation of transcriptional targets by different p53 mutants has been described in many previous studies, and can reflect a multitude of defects, including altered DNA binding activity and changes in the ability to engage with critical transcriptional cofactors or the basic transcriptional machinery.

So the main question to arise from these results must be, if not cell-cycle arrest, apoptosis, or senescence, what is p53 doing? The paper from Li et al. goes on to throw some light on this, by showing that the target genes that remain responsive to the lysine mutant of p53 include those related to the regulation of energy metabolism and antioxidant function. Specifically, the ability of 3KR to activate GLS2 (the mitochondrial glutaminase) and TIGAR (a fructose2,6 bisphosphatase) expression are highlighted in the study, although this p53 mutant is certain to retain much broader transcriptional function in both promoting and inhibiting gene expression—as illustrated by the continued ability of the mutant p53 to restrain expression of the GLUT3 glucose transporter. TIGAR (Bensaad et al., 2006) and GLS2 (Suzuki et al., 2010) contribute to what have—until now—been much less well-studied functions of p53 in modulating mitochondrial respiration and limiting both glycolysis and levels of reactive oxygen species (ROS). These functions of p53 help to oppose the Warburg effect and protect cells from oxidative stress—with loss of p53 shifting cells to adopt aerobic glycolysis so commonly seen in cancers (Puzio-Kuter, 2011). Intriguingly, mice expressing the 3KR mutant retain the ability to regulate glucose uptake, glycolysis and ROS levels—strongly suggesting that these activities may be key to the ability of p53 to limit cancer progression.

The role of increased glycolysis and ROS in cancer progression is not completely clear—and the concept that ROS can contribute to cancer progression is balanced by numerous studies showing that increased ROS can also be tumor suppressive. Nevertheless, when considering these responses in the context of p53, it seems likely that they are important components of the tumor suppressor arsenal. Clearly it remains possible that there are other functions of p53 (including the regulation of other target genes) that are retained in the lysine mutant—and these may be the critical components in tumor suppression. A careful analysis of the transcriptional program regulated by wild-type and the mutant p53 proteins will be extremely informative.

More directed studies to examine specifically the glycolytic and antioxidant functions of p53 would also be needed to fully verify their importance. However, this may also be less facile than initially expected. Although the regulation of genes like GLS2 and TIGAR by p53 could help prevent cancer development, it has also been suggested that expression of such proteins uncoupled from p53 could ultimately assist in tumor development, again underlining the paradox that modulation of glycolysis or ROS could both enhance and prevent cancer progression depending on context and level. But the identification of the responses that are truly crucial for p53-driven tumor suppression will pave the way for the development of new therapeutic approaches based on the modulation of these pathways, rather than the regulation of p53 itself. Given that over half of all human cancers have mutations in p53, the possibility of targeting cancers downstream of the defect in p53 is extremely attractive.

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Target Practice: Modeling Tumors with Stem Cells

Kai Liu[1] and Sheng Ding[1,*]

[1]Gladstone Institute of Cardiovascular Disease and Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA 94158, USA

*Correspondence: sheng.ding@gladstone.ucsf.edu

DOI 10.1016/j.cell.2012.05.019

A variant neoplastic line of human pluripotent stem cell (hPSC) displays unique tumorigenic properties, including enhanced self-renewal and survival, and aberrant blockade in differentiation. Sachlos et al. adopted a neoplastic hPSC differentiation platform to screen small molecules that selectively induce differentiation of cancer stem cells.

Recent studies support a hypothesis in which subpopulations of tumor cells, tumor initiating cells (TICs), drive tumorigenesis and give rise to a large population of differentiated progenies that make up most of a tumor. Although conventional chemotherapies reduce the bulk of the tumor by effectively eliminating highly proliferative cancer cells, refractory TICs allow tumors to recur and thus account for many treatment failures. The drug resistance of TICs may be due to their restricted cell cycle and quiescence; higher expression of drug pumps; and/or enhanced antioxidative, antiapoptotic, DNA repair, and self-renewal mechanisms, many of which are also shared by tissue-specific normal stem cells. Consequently, differentially targeting TICs, while sparing normal stem cells, is a major challenge. However, one strategy might address that challenge. TICs with oncogenic molecular alterations may be more addicted to those above enhanced stem cell mechanisms and therefore targeting such deregulation may sensitize TICs to treatments (Frank et al., 2010). For example, shifting enhanced self-renewal to the normal level may effectively render TICs more susceptible to conventional therapy.

Several studies used chemical genetics approaches to model the differentiation of stem cells and TICs. Chemical libraries were screened in hESCs (Desbordes et al., 2008) or cancer cells with certain stem-like properties (Gupta et al., 2009) to identify compounds that maintain or inhibit their self-renewal, providing tools to interrogate underlying mechanisms. However, drug discovery for differentially targeting TICs (Shen et al., 2004) has been hindered by difficulties in homogenously expanding and maintaining rare TICs in vitro (Figure 1A). This problem was addressed by developing specific conditions for stably expanding certain subpopulation of TICs (Pollard et al., 2009).

In an interesting report published in this issue of *Cell,* Sachlos et al. (2012) provide another attractive approach. They interrogated neoplastic human pluripotent stem cell (hPSC) as a human TIC surrogate for high-content screening of differentiation inducing agents (Figure 1B). Compounds identified with this model were further shown to selectively decrease the number of human CD33+ hCD45+ acute myeloid leukemia (AML) cells in a xenotransplantation model. This demonstrates the feasibility of finding therapeutic candidates for differentially targeting TIC differentiation and therapeutic potentials of such strategy for treating cancer.

Neoplastic hPSC is a culture-adapted variant hESC line (Werbowetski-Ogilvie et al., 2009) with subkaryotypic abnormalities that exhibits acquired tumorigenic features, including enhanced self-renewal with reduced growth factor dependence and blocked differentiation. Its FGFR1 and IGFR1 coexpression pattern is similar